

# Anthropogenic waste products as preferred nest sites for *Myrmica rubra* (L.) (Hymenoptera, Formicidae)

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## Abstract

Sites containing anthropogenic waste products or dumps are currently treated as refugia by a lot of animal groups, including ants. In this study the population structure of *Myrmica rubra* (L.) ants was compared between habitats containing and lacking anthropogenic waste products. The density of colonies and individual nests, number of queens and of workers both per colony and per nest, and queen size were analyzed. Twenty plots with waste products and 20 control (natural) plots (paired, each 10×10m in size) were established and compared. Results show a significant increase in colony and nest density on the plots with waste compare to the control plots. However, the number of workers, queens and queen size did not differ significantly between plot types. Overall number, as well as proportion of polycalic colonies was higher on plots with waste products. These results suggest that when *M. rubra* has more potential nest site opportunities created by human activity, the density of its colonies increases. Moreover, the anthropogenic waste product are strongly preferred nest sites for this species on the human-disturbed plots, as only them were used as nest loci there in contrast to control plots.

## Keywords

ants, colony structure, human pressure, modified habitat, polydomy, rubbish, nesting habit

## Introduction

Human-origin waste dumps are considered to be potential sources of pollution of water, soil and air, and cause the spread of parasites and of invasive animal and plant species (Ružičková et al. 1996). However, for some groups of animals, these waste dumps act as refugia (Oro et al. 2013, Baranová et al. 2015). Among these animals, important inhabitants of such sites include ants (Wieżik 2006).

Ants are ecologically very important insects, which have considerable and varied impacts on ecosystems (Wilson and Hölldobler 2005). They are very widespread, abundant and have a great influence on soil formation (Hölldobler and Wilson 1990, Folgarait 1998, Frouz and Jilková 2008). Ants also have great adaptive capacity when it comes to getting food resources. They are rather opportunistic and general predators and can modify their diet according to available resources (Wilson and Hölldobler 2005) and are also important herbivores (Rico-Gray and Oliveira 2007). Some species become domestic pests (Klotz et al. 2008), while others play an important role in conservation biology of other, threatened species of insects, such as butterflies (Buszko 2004).

Although the structure of ant colonies and their response to potential harmful human activities has received a lot of attention (e.g. Majer 1983, Krzysztofiak 1991, Pęta 1994, Holec and Frouz 2005), there is no study which would test for the effects of anthropogenic waste products as potential suitable nest sites on native ant populations to our best knowledge.

Apart from the size of colonies, the ants can react to the environmental factors such as temperature (Bishop et al. 2016) or latitude (connected with temperature) (Heinze et al. 2003) also by changing their morphology, including their body size. Taking this into account, it is good to investigate whether different density and colony structure may affect ant size.

The aim of the current study was to (a) compare the density of competing colonies, as well as individual nest density of *Myrmica rubra* (L.), a locally common ant species in Central Europe, between places containing and lacking anthropogenic waste products, (b) test if population structure (size of colonies, number of workers and of queens per colony and per nest) is different between these two habitats and (c) compare the size of queens from the two habitats in order to detect any tendencies in gyne size related to potential different densities.

This was based on hypotheses that 1) the density of colonies on plots containing anthropogenic waste products is higher, as those product can be used as nest sites and 2) the structure of colonies in both habitats is different (e.g. number of queens or workers, and the body size of queens, per colony and per nest is lower on control plots).

## Materials and methods

### Study species

*Myrmica rubra* is the most ecologically tolerant of all European *Myrmica* Latr. (Czechowski et al. 2012). It is found in a variety of habitats, as long as they are at

least partially humid. The species is also commonly found in anthropogenic habitats (Czechowski et al. 2012, Radchenko et al. 2004, Radchenko and Elmes 2010), where it sometimes uses anthropogenic waste products as nest sites (M. Michlewicz, personal observation). *Myrmica rubra* forms colonies which are generally polygynous, sometimes polycalic and consist of up to 10,000 workers (Czechowski et al. 2012). Klotz et al. (2008) even reported colonies consisting of 20,000 workers and 600 queens. The number of nests in polycalic colonies of *M. rubra* can be large and are then called “super-colonies” (Radchenko and Elmes 2010). Because of this, aggression tests, following Roulston et al. (2003), were carried out to check how many distinct colonies were present, including possible multi-nest colonies.

## Study sites

This study was undertaken in NW Poland in 2014 and 2015. Locations where study plots were established were divided into two categories: 1) “control” (20 plots) and 2) containing anthropogenic waste products (20 plots). Each plot was, according to the methods reviewed in Pętal and Pisarski (1966), 10×10m in size. Each locality has one pair of the plots – one control and one plot with waste products, at least 50 metres apart (with a maximum of 57 metres). “Experimental” plots were those considered suitable for *M. rubra* and which contained anthropogenic waste products on the ground (rubbish, empty bottles, cans, tires, clothing, rubble etc.) covering about 1/5 of the plot area (i.e. 20 m<sup>2</sup>). Natural plots were generally similar including vegetation and soil structure, but lacked any anthropogenic waste products. All plots were humid grassland with whole area sparsely, but evenly covered with not very high bushes (up to ca. 3 metres) of several plant species: *Salix* sp., *Pinus sylvestris* L., *Sambucus nigra* L., *Prunus serotina* (Ehrh.). Herbaceous vegetation consist mostly of grasses [mainly *Poa* cf. *trivialis* L. and *Elymus repens* (L.)], with some herbs present, like *Perasites hybridus* (L.), *Urtica dioica* L., *Chelidonium majus* L. and others. Each plot with waste products was previously known to the first author, with a record of that rubbish has been laying there for at least three years before this study took place. Localizations, coordinates, altitude, date of examination as well as general habitat description of each of 20 localities is available in the Suppl. material 1.

## Sampling techniques

Search was made for all nests of *M. rubra* at each site by the same methods, and in a similar time for each pair of the plots, to avoid seasonal differences. This species is relatively easy to identify in the field by an experienced person using a hand lens. In the field a hand lens with 10× magnification was used. Identification was subsequently confirmed in the laboratory using a stereomicroscope. Each nest was marked with small flag on which was written a unique number. Aggression tests were then carried out and actual colonies were re-marked with numbers if necessary. Aggression tests were carried

out in the field, using plastic Petri dishes (90 mm in diameter), cleaned with alcohol after each test. The arenas containing different colonies were marked with different colors. The assays include 1-1 and 5-5 battles of live worker specimens. Ants presenting aggressive behavior (biting and/or stinging) were marked as members of different colonies. Each time control test, with members of the same colony, was also carried out, but no aggressive behavior were observed in these. Whole number of workers from assays were taken in account when number of workers per colony was estimated. Every colony/nest was then excavated using a shovel and put on a white sheet (ca. 150×150 cm), then every ant from the nest was collected using an aspirator and put in a vial (or vials) containing 75% ethanol. In the laboratory the ants were counted and queens measured.

## Measurements

Queen measurements were taken using Zeiss AxioCam MRc 60 N-C 2/3" 0.63× camera fitted with Zeiss SteREO Lumar.V12 stereomicroscope and AxioVision rel. 4.8 software. Measurements were taken on the base of photographs using the given software. Mesosoma length (ML) was measured as a surrogate of body size, as it is strongly correlated with queen size (Steiner et al. 2006). General aim of taking measurements was to detect any tendencies in queen size, potentially related to the different nest densities. Sixty queens from control and 60 from plots with waste products were measured, selected randomly from the whole number of queens collected from all nests.

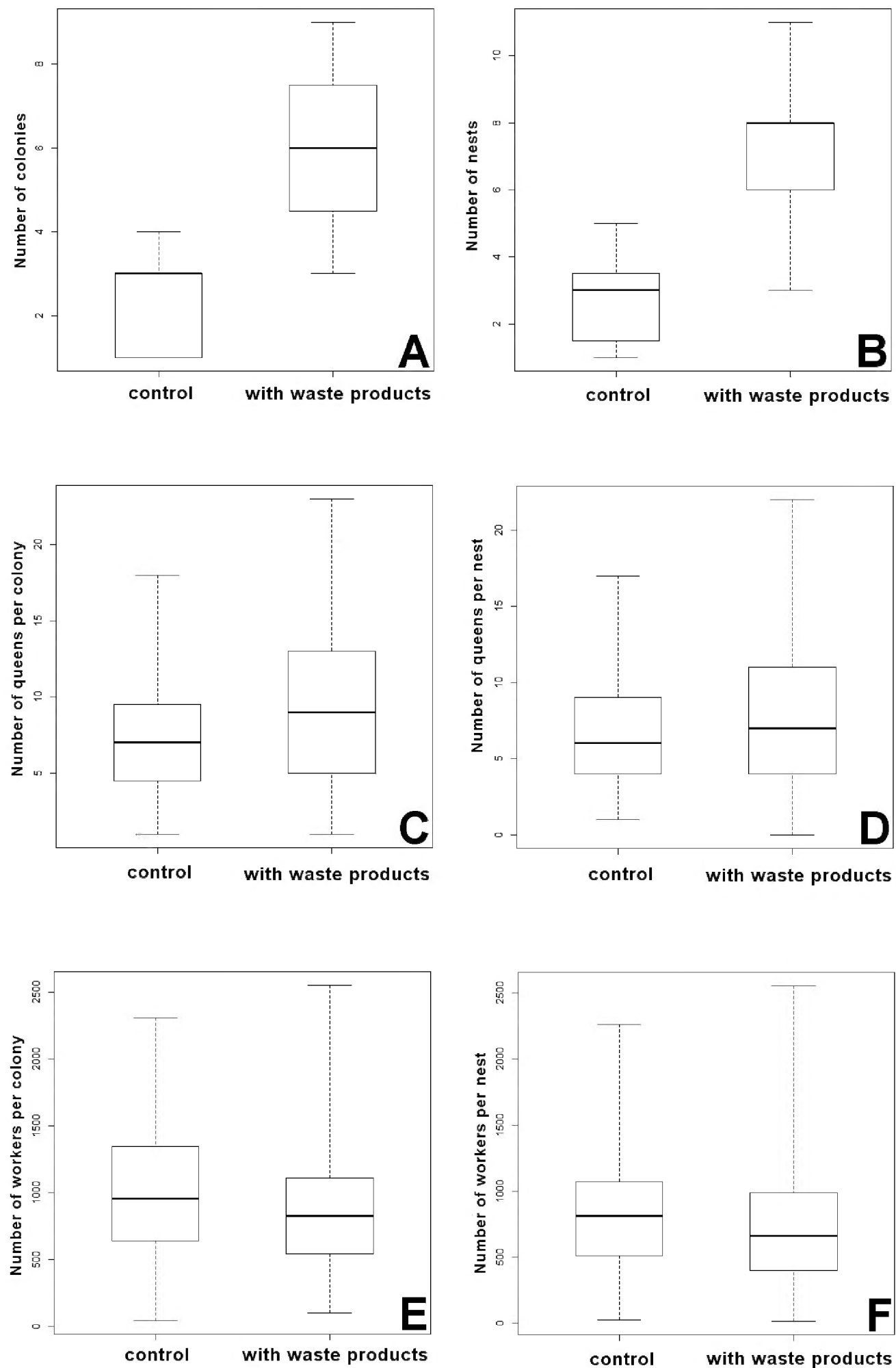
## Statistical analysis

The results were analyzed using PASW® Statistics 18 and RStudio version 3.2.3 software (R Core Team 2015). First, Shapiro-Wilk normality test was used to check normality of distributions of the variables, and because some of them have non-normal distribution a non-parametric Wilcoxon signed-rank test (comparing the effects among the paired plots) and Mann-Whitney U-test (comparing the queen size) were used. Chi-square test was used to compare proportion of polycalic colonies among the two plot kinds.

## Results

### Colony and nests densities

Total number of 166 colonies (47 on control and 119 on experimental plots) consisting of a total number of 196 nests (53 on control and 143 on experimental plots) was found. Statistical analysis using a Wilcoxon signed-rank test found a significant differences between both the number of colonies (Fig. 1A;  $W = 16$ ,  $P < 0.00001$ ) and nests (Fig. 1B;  $W = 13.5$ ,  $P < 0.00001$ ) between control and plots with waste products.



**Figure I.** Variation of measured nest parameters between control plots and plots with waste products in *Myrmica rubra*. **A** colony density ( $P < 0.0001$ ) **B** nest density ( $P < 0.0001$ ) **C** number of queens per colony ( $P = 0.06$ ) **D** number of queens per nest ( $P = 0.26$ ) **E** number of workers per colony ( $P=0.32$ ) **F** number of workers per nest ( $P = 0.06$ ). Numbers are given as median per plot (per 100 m<sup>2</sup>), whiskers of the boxplot represent the range of minimum and maximum values.

Overall, 26 polycalic colonies was found (5 on control and 21 on experimental plots), and chi-square test found significant difference in proportion of polycalic colonies among the two plot kinds ( $\chi^2 = 4.6493$ ,  $P = 0.03$ ). Minimum number of nests in those colonies was two, while the maximum was four (one time, on plot with waste products). The mean number of colonies per plot (100 m<sup>2</sup>) on control areas was  $2.35 \pm 0.23$  (and the mean number of nests  $2.65 \pm 0.29$ ) and on plots with waste products  $5.95 \pm 0.4$  for colonies and  $7.25 \pm 0.44$  for nests.

## Colony structure and size

### Number of queens

In total, 1479 queens were sampled (359 from control and 1120 from plots with waste products). No “microgyne” forms were found in any nest (i.e. recognized by eye). The difference between number of queens on control and “experimental” plots both per colony (Fig. 1C) and per nest (Fig. 1D) was statistically insignificant ( $W = 2269.5$ ,  $P = 0.06$  and  $W = 3438.5$ ,  $P = 0.26$ , respectively), although there was a tendency of a higher queen numbers recorded in the plots with waste products. Mean queen number per colony was  $7.6 \pm 0.6$  (control) and  $9.4 \pm 0.47$  (plot with waste products), and per nest was  $6.8 \pm 0.53$  (control) and  $7.7 \pm 0.39$  (plot with waste products).

### Number of workers

The difference between number of workers both per colony (Fig. 1E) and nest (Fig. 1F) on both plot types was statistically insignificant ( $W = 3076$ ,  $P = 0.32$  and  $W = 2269.5$ ,  $P = 0.06$ ), although the numbers were higher on average in the control plots this time. Mean number of workers per colony on control plots with waste products was  $1024.3 \pm 85.27$  and  $937.5 \pm 51.35$ , respectively. Values for nest were  $908.3 \pm 76.67$  (control) and  $769.1 \pm 44.82$  (plots with waste products).

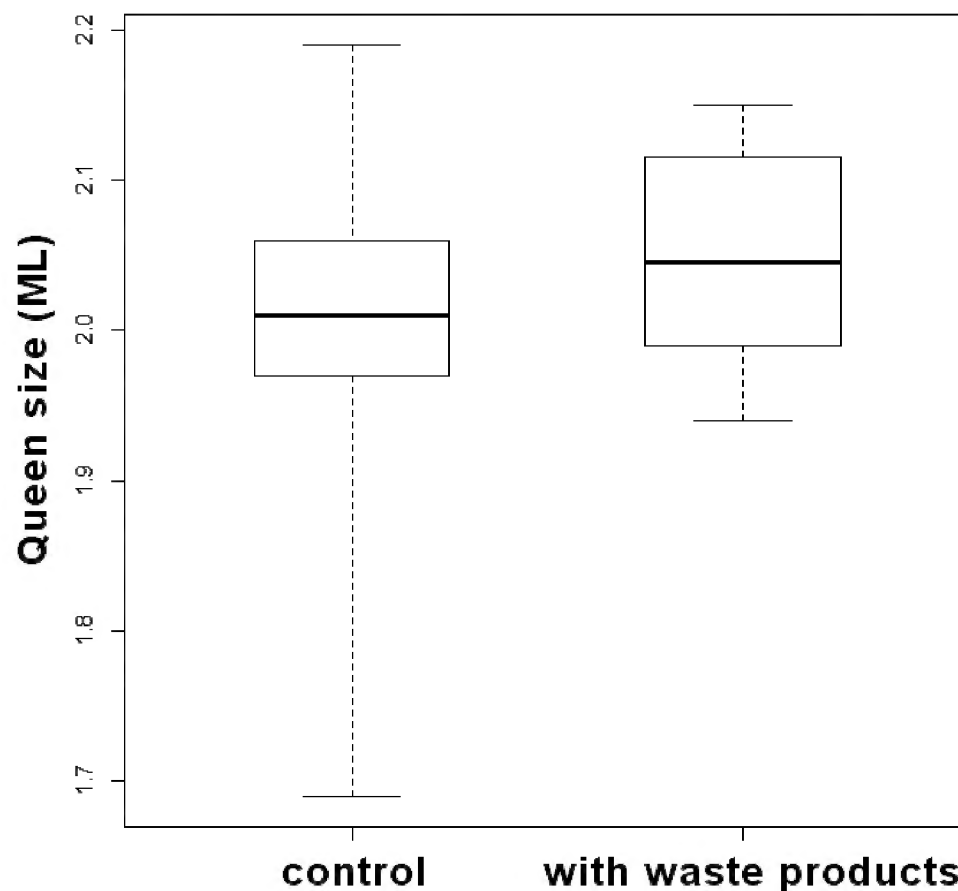
### Queen measurements

The difference in size (based on mesosoma length) between queens from control ( $n = 60$ ) and experimental ( $n = 60$ ) plots was not statistically significant (Mann-Whitney U-test,  $Z = -0.19$ ,  $P = 0.8493$ ) (Fig. 2). Mean size of ML of measured queens was  $2.02 \pm 0.01$  mm on control and  $2.05 \pm 0.01$  mm on plots with waste products.

### Nest sites

Interestingly, on plots with waste products only these microhabitats were used as nest sites by ants. These include: empty bottles (both plastic and glass) and cans, jars, tires, clothing, bricks with holes, old carpets, plastic carrier bags, other pieces of metal or plastic, often partially covered with wet soil. On control plots *M. rubra* nests were always found in clumps of grass and/or under rocks or pieces of wood, but those microhabitats were not occupied by the species in plots with the waste.





**Figure 2.** Variation in median queen size (mesosoma length in mm) between control plots and plots with waste products in *Myrmica rubra*. Difference is statistically insignificant. Whiskers of the boxplot represents the range of minimum and maximum values.

## Discussion

Previous studies show that in suitable humid grassland ecosystems the density of *M. rubra* nests is limited more by the number of appropriate nest sites than by the availability of food resources. When one nest site is, for some reason, abandoned by one colony, it is quickly recolonized by another of (in most cases) the same *Myrmica* species (Radchenko and Elmes 2010).

Concerning densities of *M. rubra*, only grassland habitats with no substantial volume of waste products have been studied before (e.g. Parapura and Pisarski 1971, Pętal 1980, Seppä and Walin 1996, Radchenko and Elmes 2010 and references therein, Huszár et al. 2014). Our effort is, to our knowledge, hence the first comparing nest and colonies densities, as well as workers and queens numbers (and size of the latter), on plots with and without anthropogenic waste product in order to investigate, whether those pieces of rubbish may be used by ants as nest sites and affect their populations. We showed, that anthropogenic waste products (listed in Results) can act as preferred nest loci. On plots with high level of anthropogenic waste products, only those are used as nest sites. However, increase of food resources (especially a higher quantity of small invertebrates there) may also probably affect the establishment and survival of the ant colonies in habitats with anthropogenic waste products (M. Michlewicz, personal observation), although we did not measure these parameters in our

plots. Among studied variables, only number of colonies and number of nests on both plot types showed significant differences. Previous studies, also conducted in Poland (Czechowski et al. 2012 and references therein) showed, that densities of *M. rubra* nests vary greatly depending on habitat type. Therefore, it is crucial to properly choose both “experimental” and control plot within the same habitat and time to control, also for microenvironmental and seasonal characteristics, as done in this study.

To our knowledge, highest density of *M. rubra* in Poland was reported in the Carpathian alder forest in Pieniny National Park and equals 100 nests per 100 m<sup>2</sup> (Czechowska 1976). Some other studies also report rather high densities, e.g. 26.0 (Parapura and Pisarski 1971),  $20.5 \pm 19.3$  (Babik et al. 2009) and 20.0 (Czechowski 1985). Although, some studies did not show such high densities, e.g.  $1.0 \pm 0.31$  (mean  $\pm$  SD) (Rzeszowski et al. 2013), 6.0 (Czechowski et al. 1990), 0.05, 3.0 and 14.0 (Pętał 1980) (all given in number of nests per 100 m<sup>2</sup>). Despite the fact that our results from plots with waste products (mean  $7.25 \pm 0.44$  nests/100 m<sup>2</sup>) are not as high as some literature data, this density significantly varies from results from control plots (mean  $2.65 \pm 0.29$  nests/100 m<sup>2</sup>). Notably, the “control-experimental” plot pairs were chosen in the very similar habitats (see Suppl. material 1), but only waste products were used as nest sites in the latter plots. This is a strong evidence that the increased nest and colony densities observed here were caused by the human-deposited waste, and that *M. rubra* ants prefer these waste products as nest sites, whenever available.

As some studies document, number of queens both per colony and per nest, as well as number of workers can also widely differ in *Myrmica* ants. Seppä and Walin (1996) report colonies consisting of mean number of 7.56 queens and 961–828 workers. Elmes (1980) reports mean number of 15.89 queens per colony and Elmes and Pętał (1990) write about overall number of workers per colony between 1,000 and 2,000. Our results on both worker and queen numbers fit those ranges. However, differences in those numbers between both plot types were insignificant. This might indicate, that living in higher densities makes *M. rubra* ants more tolerant to other colonies of the same species, but those colonies does not have to be necessary smaller (both in respect the number of workers and queens). This is in agreement with our observation of also more polycalic colonies to be established on the plots with waste products, as those colonies act behaviorally and ecologically as a single nest.

Differences in queen size between the two plot types were not statistically significant. Similar results were found by Huszár et al. (2014). This shows that living at higher density probably does not necessarily lead to changes in gyne morphology. Moreover, no “microgyne” form queens were found during the study process. However, although the queen size did not differ, there was slight tendency (insignificant) to a higher number of queens but lower number of workers per nest in the plots containing waste, which is in agreement with strategy of the ants to invest more to sexuals and to establishment of the new nest satellites, where enough “empty” nest sites is available (Oster and Wilson 1978, Poitrineau et al. 2009).

Some research showed, that where *M. rubra* forms polycalic nest systems, the number of other species of ants is significantly lower (Seppä and Pamilo 1995, Seppä and



Walén 1996, Huszár et al. 2014). Future studies are needed to test if similar results will be found on habitats where *M. rubra* live in higher density because of presence of anthropogenic waste products and consider also the effects of its population increase on the other ant species. Such further insight is needed given the ongoing damage of the natural environments by humans, which can influence, however, positively some insect populations, as demonstrated here for the case of *M. rubra* and waste products.

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## **Supplementary material I**

### **Study sites**

Authors: Michał Michlewicz, Piotr Tryjanowski

Data type: species data

Explanation note: General description of study localities, including the dates of investigation.

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